盛岡高等農林學校

學術報告

第八號

THE BULLETIN

OF THE

IMPERIAL COLLEGE OF AGRICULTURE AND FORESTRY

MORIOKA,

JAPAN

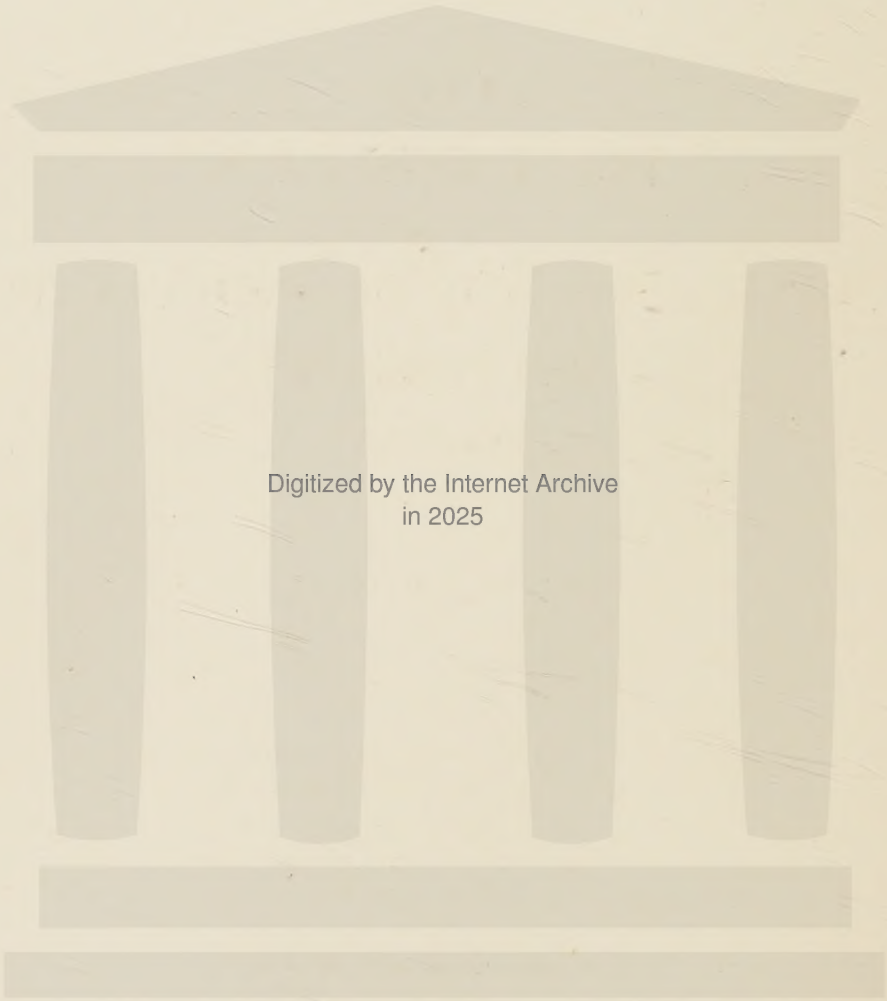
No. VIII.

SUPPLEMENTARY NOTE ON THE ENZYME ACTIVITY OF RHIZOCTONIA SOLANI KÜHN. PHYSIOLOGICAL STUDIES ON GLOEOSPORIUM WITH SPECIAL REFERENCE TO THE STRAINS OBTAINED FROM APPLE AND CHERRY.

TAKASHI MATSUMOTO

大正十三年九月

MORIOKA, SEPTEMBER, 1924.



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SUPPLEMENTARY NOTE ON THE ENZYME ACTIVITY OF RHIZOCTONIA SOLANI KÜHN

TAKASHI MATSUMOTO

In my previous paper ('23) I expressed my opinion concerning the effect of H-ion concentrations on the activity of some enzymes, but some of my conclusions stated in that paper seemed to base upon insufficient data in some instances, therefore it is considered necessary to present here some additional data as well as some new interpretations on this subject.

Methods of Experiment

In studying extracellular enzymes, filtrates of the culture solutions on which the fungus in question has grown are used. For intracellular enzymes the dry fungus mats obtained from the above mentioned culture solutions are powdered in a mortar and again ground with a small amount of doubly distilled water and then diluted to such a concentration as desired. The mixtures are kept at 30°C. for 12 hours and filtered.

A range of pH value is obtained by additions of regulated amounts of acid or alkali and doubly distilled water according to the manner described in the following tables. All the solutions are placed in a thermostat ranging from 29° to 30°C. At the end of 30 hours, the hydrolysing power of the enzymes is determined by modified Bertrand method as des-

Matsumoto, T. ('23) Further Studies on Physiology of Rhizoctonia Solani Kuhn. Bull. Imp. Coll. Agr. and For. Morioka, No. 5. 64 p. 1923.

Duggar, B. M., J. W. Severy and H. Schmitz. ('17) Studies in the Physiology of the Fungi. The growth of certain fungi in plant decoctions. Ann. Mo. Bot. Gard. 4:169–173, 279–288, 1917.

cribed later. Two cc. of each of the solutions are accurately pipetted into each of two 15 cc. centrifuge tubes and made neutral to phenolphthalein by adding either NaOH or HCl. Five cc., or sometimes 10 cc., of Fehling's solution are added to each of these tubes at a time, and the tubes are immersed immediately in an active boiling water for 10 minutes. The amount of reduction is determined by using 0.5% $KMnO_4$.

1. Diastase activity. The fungus has grown on carrot decoction prepared in the manner described by Duggar, Severy and Schmitz ('17). Fifty cc. of each of the culture solutions having an initial concentration of pH 4.6 and pH 6.0 are pipetted into each of 150 cc. Erlenmeyer flasks, which are inoculated with P1 and then placed in a thermostat ranging from 29° to 30°C.

A range of pH value is obtained according to the method described in the following table.

TABLE I.

AMOUNT OF MATERIALS USED FOR PREPARING SOLUTIONS.

No.	Cc. of N/10 HCl	Cc. of N/10 NaOH	Cc. of Water	Cc. of 2% Starch	Cc. of Enzyme
1	1.2	0	12.80	10	6
2	0.6	0	13.40	10	6
3	0.2	0	13.80	10	6
4	0	0	14.00	10	6
5	0	0.2	13.80	10	6
6	0	0.6	13.40	10	6

Series I and II are set up by adding the enzymes obtained from the culture media having the above mentioned two different H-ion concentrations, i.e. pH 4.6 and pH 6.0 respectively. Control A is set up by adding boild enzymes instead of unboiled, and control B is made by addition of doubly distilled water instead of the starch solution. For intracellular diastase 1% mycelial extraction is used. Determination of enzyme activity is made at two different times; namely the

first determination is made after 7 days' growth of mycelium, and the second is made after 14 days' growth. Average dry weight of mycelium obtained from three flasks will be shown as follows:

TABLE II.

GROWTH OF MYCELIUM IN GRAM.

Series	After 7 days	After 14 days
Series I	0.205	0.429
Series II	0.155	0.345

To each of these solutions one per cent of toluol is added as an antiseptic. The result of the experiment will be tabulated as follows:

TABLE III.

SHOWING THE ACTIVITY OF INTRA-AND EXTRACELLULAR DIASTASE.

Incubation period	Enzyme	No.	Series I						
			Total		Control A		Control B		Enzyme activity
			pH	$KMnO_4$	pH	$KMnO_4$	pH	$KMnO_4$	
After 7 days' incubation	Extracellular	1	3.6	0.80	3.6	0.65	3.6	0.60	0.15
		2	4.3	0.80	4.3	0.50	4.3	0.50	0.30
		3	5.0	1.00	4.9	0.55	4.9	0.50	0.45
		4	5.6	1.10	5.5	0.55	5.5	0.50	0.55
		5	6.6	1.30	6.2	0.55	6.5	0.55	0.75
		6	9.1	0.70	9.0	0.50	9.0	0.50	0.20
	Intracellular	1	3.5	0.20	3.5	0.15			0.05
		2	4.1	0.25	4.0	0.15			0.10
		3	5.1	0.25	5.1	0.10			0.15
		4	6.7	0.40	6.7	0.10			0.30
		5	7.4	0.35	7.4	0.10			0.25
		6	8.5	0.20	8.5	0.10			0.10
After 14 days' incubation	Extracellular	1	3.8	0.40	4.0	0.20	4.2	trace	0.20
		2	5.8	0.90	5.8	0.20	5.4	,,	0.70
		3	6.8	1.10	6.8	0.15	6.8	,,	0.95
		4	7.7	0.90	7.7	0.15	7.7	,,	0.75
		5	8.8	0.50	8.8	0.20	8.8	,,	0.30
		6	9.5	0.15	9.5	0.10	9.5	,,	0.05
	Intracellular	1	3.4	0.20	3.4	0.15			0.05
		2	4.2	0.30	4.2	0.15			0.15
		3	5.0	0.40	5.0	0.10			0.30
		4	6.6	0.55	6.7	0.10			0.45
		5	7.4	0.55	7.4	0.10			0.45
		6	8.4	0.25	8.4	0.10			0.15

Incubation period	Enzyme	No.	Series II						
			Total		Control A		Cantrol B		Enzyme activity
			pH	$KMnO_4$	pH	$KMnO_4$	pH	$KMnO_4$	
After 7 days' incubation	Extracellular	1	4.0	0.80	4.0	0.65	4.0	0.60	0.15
		2	4.5	0.80	4.5	0.60	4.5	0.60	0.20
		3	5.4	1.00	5.4	0.65	5.4	0.65	0.35
		4	6.2	1.10	6.2	0.65	6.2	0.65	0.45
		5	6.8	1.15	7.0	0.60	6.8	0.60	0.55
		6	9.2	0.70	9.2	0.60	9.0	0.60	0.10
	Intracellular	1	3.4	0.20	3.5	0.15			0.05
		2	4.3	0.25	4.3	0.15			0.10
		3	5.2	0.30	5.2	0.10			0.20
		4	6.7	0.45	6.8	0.10			0.35
		5	7.5	0.40	7.5	0.10			0.30
		6	8.5	0.25	8.5	0.10			0.15
After 14 days' incubation	Extracellular	1	4.1	0.50	4.0	0.20	4.0	trace	0.30
		2	5.8	1.00	5.8	0.25	5.7	0.15	0.75
		3	6.8	0.90	6.7	0.15	6.7	,,	0.75
		4	7.8	0.70	7.8	0.15	7.8	,,	0.55
		5	9.0	0.35	9.0	0.15	9.0	,,	0.20
		6	9.6	0.10	9.6	0.10	9.6	,,	0
	Intracellular	1	3.4	0.30	3.5	0.20			0.10
		2	4.2	0.45	4.2	0.20			0.25
		3	5.0	0.65	5.0	0.15			0.50
		4	6.6	0.70	6.6	0.10			0.60
		5	7.6	0.70	7.6	0.15			0.55
		6	8.5	0.30	8.5	0.10			0.20

2. *Invertase activity. Experiment 1.* Potato decoction is prepared in the manner described by Duggar, Severy and Schmitz ('17). Twenty five cc. of each of the culture solutions having an initial H-ion concentration of pH 4.6 and pH 6.8 are pipetted into each of 150 cc. Erlenmeyer flasks, which are inoculated with P1 and then placed in a thermostat ranging from 29° to 30°C. Series of the solutions is prepared in the same manner as described in the preceding experiment, but using two per cent of sucrose instead of the starch solution. Average dry weight of mycelium obtained from three flasks will be shown in the following table.

TABLE III.

SHOWING THE GROWTH OF MYCELIUM IN GRAM.

Series	After 7 days	After 14 days
Series I	0.045	0.068
Series II	0.035	0.060

The result of the experiment will be tabulated as follows:

TABLE IV.

SHOWING THE ACTIVITY OF INTRA-AND EXTRACELLULAR INVERTASE.

Incubation period	Enzyme	No.	Series I						Series II					
			Total		Control A.		Control B	Enzyme activity	Total		Control A		Control B	Enzyme activity
			pH	$KMnO_4$	pH	$KMnO_4$	$KMnO_4$		pH	$KMnO_4$	pH	$KMnO_4$	$KMnO_4$	
After 7 days' incubation	Extracellular	1	3.0	2.65	2.8	0.40	trace	2.25	3.5	2.65	3.3	0.35	trace	2.30
		2	5.2	2.45	5.0	0.15	,,	2.30	5.2	2.50	5.0	0.15	,,	2.35
		3	6.6	2.65	6.6	0.10	,,	2.55	6.8	2.60	6.8	0.10	,,	2.50
		4	8.0	2.65	8.0	0.10	,,	2.55	8.2	2.55	8.2	0.10	,,	2.45
		5	8.9	2.60	9.0	0.10	,,	2.50	8.8	2.55	9.0	0.10	,,	2.45
		6	9.4	1.30	9.5	0.10	,,	1.20	9.4	1.35	9.5	0.10	,,	1.25
	Intracellular	1	3.5	0.85		0.35		0.50	3.5	0.90	3.3	0.35		0.55
		2	4.5	0.95		0.25		0.70	4.5	0.95		0.20		0.75
		3	5.1	0.95		0.20		0.75	5.2	1.00		0.20		0.80
		4	6.6	0.90		0.10		0.80	6.6	0.95		0.10		0.85
		5	7.9	0.85		0.10		0.75	8.0	0.90		0.10		0.80
		6	8.9	0.80		0.10		0.70	8.9	0.80		0.10		0.70
After 14 days' incubation	Extracellular	1	3.2	3.00	3.2	0.40	trace	2.60	3.7	2.80	3.7	0.40	trace	2.40
		2	5.3	2.75	5.2	0.15	,,	2.60	6.2	2.65	6.2	0.15	,,	2.50
		3	6.8	2.70	6.8	0.10	,,	2.60	6.8	2.70	6.8	0.10	,,	2.60
		4	8.0	2.60	8.0	0.10	,,	2.50	8.2	2.70	8.1	0.15	,,	2.55
		5	9.0	2.50	9.2	0.10	,,	2.40	9.0	2.55	9.2	0.10	,,	2.45
		6	9.6	1.20	9.6	0.10	,,	1.10	9.6	1.55	9.6	0.10	,,	1.45
	Intracellular	1	3.4	1.00		0.35		0.65	3.4	1.05		0.30		0.75
		2	4.2	1.10		0.25		0.85	4.2	1.25		0.25		1.00
		3	5.0	1.10		0.20		0.90	5.0	1.25		0.20		1.05
		4	6.6	1.05		0.10		0.95	6.6	1.25		0.15		1.10
		5	7.3	1.00		0.10		0.90	7.6	1.10		0.10		1.00
		6	8.4	0.90		0.10		0.80	8.8	0.95		0.10		0.85

Experiment 2. Mycelial extraction is made from the mats of the mycelium growing on Czapek's solution which has been incubated for 3 weeks at 29°C. The solutions used for the experiment is prepared in the manner as described in the following table.

TABLE V.

AMOUNTS OF MATERIALS USED FOR PREPARING SOLUTIONS.

No.	N/5 HCl	N/5 NaOH	Water	4% Sucrose	1% Enzyme
1	1.00	0	4.00	5	5
2	0.80	0	4.20	5	5
3	0.60	0	4.40	5	5
4	0.40	0	4.60	5	5
5	0.20	0	4.80	5	5
6	0.10	0	4.90	5	5
7	0.05	0	4.95	5	5
8	0	0	5.00	5	5
9	0	0.1	4.90	5	5
10	0	0.2	4.80	5	5
11	0	0.5	4.50	5	5
12	0	0.8	4.20	5	5

A control series is set up by adding 5 cc. of doubly distilled water instead of the mycelial extraction. Determination of hydrolysing power is made in the same manner as described in the preceding experiment.

The result of the experiment will be shown in the following table.

TABLE VI.

SHOWING THE EFFECT OF H-ION CONCENTRATION ON THE ACTIVITY OF INVERTASE.

No.	After 12 hours					After 24 hours			After 2 days		
	Total		Control		Enzyme activity	Cc. of $KMnO_4$		Enzyme activity	Cc. of $KMnO_4$		Enzyme activity
	pH	$KMnO_4$ cc.	pH	$KMnO_4$ cc.		Total	Control		Total	Control	
1	3.0	0.80	1.6†	0.80	0	1.80	1.30	0.50	2.70	1.90	0.80
2	3.4	0.80	1.6	0.60	0.20	1.70	1.00	0.70	2.75	1.65	1.10
3	3.6	1.05	1.9	0.70	0.35	1.60	0.90	0.70	2.55	1.40	1.15
4	3.8	1.00	2.4	0.60	0.40	1.60	0.70	0.90	2.55	1.00	1.55
5	4.0	1.10	3.0	0.40	0.70	1.60	0.40	1.20	2.75	0.65	2.10
6	4.2	1.10	3.2	0.30	0.80	1.70	0.30	1.40	2.80	0.40	2.40
7	5.2	1.25	3.4	0.20	1.05	2.10	0.20	1.90	3.20	0.20	3.00
8	6.8	1.25	6.8	0.20	1.05	1.60	0.20	1.40	2.15	0.20	1.95
9	9.2	0.55	9.6	0.20	0.35	0.95	0.20	0.75	1.20	0.20	1.00
10	9.6	0.45	9.8	0.20	0.25	0.75	0.20	0.55	1.10	0.20	0.90
11	9.8	0.30	9.8†	0.20	0.10	0.35	0.20	0.15	0.40	0.20	0.20
12	9.8†	0.20	9.8†	0.20	0	0.20	0.20	0	0.20	0.20	0

Experiment 3. A somewhat similar experiment is performed by using boiled enzymes instead of water as a control. The solutions used and the result of the experiment will be shown in the following table.

TABLE VII.

SHOWING TITRATION DATA.

	Series	N/5 HCl	N/5 NaOH	Water	4% sucrose	0.4% Enzyme	pH
1	Enzyme sol.	1.00	0	4.00	5	5	2.8
	Control	1.00	0	4.00	5	5. boiled	2.8
2	Enzyme sol.	0.60	0	4.40	5	5	3.3
	Control	0.60	0	4.40	5	5. boiled	3.4
3	Enzyme sol.	0.20	0	4.80	5	5	3.7
	Control	0.20	0	4.80	5	5. boiled	3.8
4	Enzyme sol.	0.10	0	4.90	5	5	4.0
	Control	0.10	0	4.90	5	5. boiled	4.0
5	Enzyme sol.	0.05	0	4.95	5	5	4.8
	Control	0.05	0	4.95	5	5. boiled	4.8
6	Enzyme sol.	0	0	5.00	5	5	6.6
	Control	0	0	5.00	5	5. boiled	6.6
7	Enzyme sol.	0	0.1	4.90	5	5	8.6
	Control	0	0.1	4.90	5	5. boiled	8.5

TABLE VIII.

SHOWING THE EFFECT OF H-ION CONCENTRATION ON THE ACTIVITY OF INVERTASE.

No.	After 6 hours					After 24 hours		
	Total		Control		Enzyme activity	Cc. of $KMnO_4$		Enzyme activity
	pH	$KMnO_4$	pH	$KMnO_4$		Total	Control	
1	2.8	1.10	2.8	1.10	0	2.00	1.60	0.40
2	3.3	0.50	3.4	0.40	0.10	1.60	1.10	0.50
3	3.7	0.50	3.8	0.30	0.20	1.10	0.55	0.55
4	4.0	0.55	4.0	0.30	0.25	1.05	0.45	0.60
5	4.8	0.60	4.8	0.20	0.40	1.15	0.40	0.75
6	6.6	0.70	6.6	0.20	0.50	1.10	0.30	0.80
7	8.6	0.50	8.5	0.20	0.30	0.85	0.25	0.60

3. Maltase activity. Carrot decoction is prepared in the manner described by Duggar, Severy, and Schmitz ('17). Fifty cc. of each of the culture solutions having an initial H-ion concentration of pH 4.6 and 6.0 are pipetted into each of 150 cc. Erlenmeyer flasks, which are inoculated with P_1 and then placed in a thermostat ranging from 29° to 30°C. As already stated in my previous paper ('23), maltase activity is more sparingly found in mycelial extractions, as compared with the corresponding activity in the filtrates, therefore the present experiment is carried out by using extracellular enzymes alone. The solutions are prepared in the manner as follows:

Dry weight of mycelium is read as follows: Series I. 0.325 (after 10 days), 0.450 (after 18 days); and Series II. 0.280 (after 10 days), 0.385 (after 18 days).

TABLE IX.

AMOUNTS OF MATERIALS USED FOR PREPARING SOLUTIONS.

	N/10 HCl	N/10 NaOH	Water	2% Maltose	Enzyme
1	2.0	0	12.00	10	6
2	1.2	0	12.80	10	6
3	0.6	0	13.40	10	6
4	0.2	0	13.80	10	6
5	0	0	14.00	10	6
6	0	0.2	13.80	10	6
7	0	0.6	13.40	10	6

Determination of the enzyme activity is made after an incubation period of 3 days.

TABLE X.

SHOWING THE EFFECT OF H-ION CONCENTRATION ON MALTASE ACTIVITY.*

Growth of mycelium	No.	Series I							Series II						
		Total		Control A		Control B		Enzyme activity	Total		Control A		Control B		Enzyme activity
		pH	KMnO4	pH	KMnO4	pH	KMnO4		pH	KMnO4	pH	KMnO4	pH	KMnO4	
After 10 days	2	3.8	1.75	3.8	1.55	—	0.30	0.20	4.0	1.80	4.1	1.60	—	0.30	0.20
	3	5.6	1.80	5.6	1.55	—	0.30	0.25	5.7	1.80	5.7	1.55	—	0.30	0.25
	4	6.5	1.70	6.6	1.50	—	0.25	0.20	6.6	1.70	6.7	1.55	—	0.30	0.15
	5	7.5	1.65	7.5	1.55	—	0.30	0.10	7.7	1.65	7.7	1.55	—	0.30	0.10
	6	8.2	1.60	8.2	1.55	—	0.30	0.05	8.6	1.55	8.6	1.55	—	0.30	0
	7	9.3	1.55	9.5	1.55	—	0.30	0	9.4	1.55	9.4	1.55	—	0.30	0
After 16 days	1	3.4	1.60		1.25	—	trace	0.35	3.8	1.55		1.25	—	trace	0.30
	2	5.8	1.70		1.25	—	,,	0.45	6.0	1.65		1.25	—	,,	0.40
	3	6.5	1.60		1.20	—	,,	0.40	6.8	1.60		1.30	—	,,	0.30
	4	7.0	1.40		1.25	—	,,	0.15	7.4	1.45		1.30	—	,,	0.15
	5	8.0	1.35		1.25	—	,,	0.10	8.4	1.35		1.30	—	,,	0.05
	6	9.0	1.30		1.30	—	,,	0	9.1	1.30		1.30	—	,,	0
	7	9.8	1.30		1.30	—	,,	0	9.8	1.30		1.30	—	,,	0

4. Emulsin activity. Two per cent of amygdalin is prepared, and as a source of enzyme 0.5% mycelial extraction is used. For control boiled mycelial extraction is used. The result of the experiment will be tabulated as follows:

* The experiment is conducted in the same manner as described at p. 2.

TABLE XI.

SHOWING THE EFFECT OF H-ION CONCENTRATION ON THE ACTIVITY OF EMULSIN.

pH	Cc. of 0.25% $KMnO_4$**					
	After 24 hours			After 48 hours		
	Total	Control	Enzyme Activity	Total	Control	Enzyme activity
2.1	0.90	0.60	0.30	1.10	0.65	0.45
2.5	0.90	0.60	0 30	1.10	0.70	0.40
3.2	0.90	0.60	0.30	1.20	0.65	0.55
3.4	1.20	0.60	0.60	1.20	0.60	0.60
4.0	1.10	0.60	0.50	1.20	0.60	0.60
6.2*	1.20	0.60	0.60	1.30	0.60	0.70
9.0	0.80	0.60	0.20	1.10	0.60	0.50
9.5	0.60	0.60	0	0.80	0.60	0.20

A similar experiment is performed by using the solutions having different H-ion concentrations. The result of the experiment made after 40 hours' incubation will be shown as follows :

TABLE XII.

SHOWING THE EFFECT OF H-ION CONCENTRATION ON THE ACTIVITY OF EMULSIN.

pH	Cc. of $KMnO_4$		
	Total	Control	Enzyme activity
3.8	0.70	0.30	0.40
4.0	0.70	0.30	0.40
5.2	0.90	0.30	0.60
6.2*	0.90	0.30	0.60
6.6	0.80	0.30	0.50
8.4	0.75	0.30	0.45

* Natural H-ion concentration.

** Diluted to $\frac{1}{2}$ strength.

Discussion of Data.

1. Diastase, invertase and maltase of the fungus in question accumulate more abundantly in the cultures incubated for two weeks.

2. A slight increase of accumulation of extracellular diastase occurs in the cultures having an initial H-ion concentration of pH 4.6, as compated with the same enzymes in the cultures of the higher pH exponent, i.e. pH 6.0, while the accumulation of the intracellular enzyme is more or less increased in the solutions having the higher initial pH concentration. The same is true with invertase to a certain extent excepting the extracellular in the strong alkaline solutions. It may be rather difficult to decide whether this increase of accumulation of reducing sugar in extracellular is directly due to the increase of the actual activity of these enzymes or not, because it is remembered that this accumulation is partly or wholly due to the increase of the total amounts of mycelial growth in the lower pH media. Experiments concerning this subject are still in progress, therefore a definite conclusion will be left for the future investigation.

3. An optimum zone of pH value for the extra-and intracellular diastase comes around pH 6.6 in both of the series studied.

4. A certain amount of sugar found in control B of extracellular diastase after an incubation period of 7 days may be due to the presence of the sugar remaining in the original culture solutions, and not due to the enzyme activity.

5. pH value of the culture solutions, carrot and potato decoction, is more or less raised during the growth of the fungus (more detailed data are given in p. 15-17 of my previous paper ('23)), but rate of the shifting of pH value is strikingly different in these two media. In the potato media the change of pH after 7 days' incubation is more remarkable as compared with those in the carrot media.

6. In the solutions added with sucrose hydrolysis takes place to a certain extent by additions of HCl, although this reaction is not so noticeable in the very weak solutions. Concerning this matter very little attention was paid in my previous paper ('23).

7. An optimum zone of pH value for the extra-and intracellular invertase may be somewhere around pH 6.6.

8. Invertase activity is not so strikingly reduced in the solutions having the higher pH concentrations, while diastase is more sensitive to both acid and alkali,

9. In acid side (within the limits studied) no marked difference in the effect of H-ion concentration on maltase activity is noticed, but an optimum zone seems to come around pH 5.6-6.0. In alkaline side the activity is considerably reduced.

10. So far as the present experiments are concerned the emulsin is more active at pH 5.2-6.2.

Notice.

The following sentences are to be added to my previous paper ('23).

1. "This shifting of the active acidity toward alkaline side may be partly due to the fact that the fungus liberates alkaline sodium compounds at the expense of sodum nitrate" are omitted, and followed by the following sentence. "The culture media with NH_4NO_3 have a tendency to become acidic during the growth of the fungi studied".

2. All the solutions for the study of enzymes were incubated at 29°-30°C., unless a special notice concerning the temperature requirement was given.

3. In some instances an initial was substituted for a control owing to the scarcity of materials.

4. In experiment 2 of the studies on the secretion of pectinase the control was made by using boiled filtrate instead of distilled water.

PHYSIOLOGICAL STUDIES ON GLOEOSPORIUM WITH SPECIAL REFERENCE TO THE STRAINS OBTAINED FROM APPLE AND CHERRY

TAKASHI MATSUMOTO

There has always been a great difficulty in determining the form of *Gloeosporium*, especially the forms from apple have been very perplexing to many investigators. Edgerton ('08) called attention that there seemed to be two different strains of the apple anthracnose in America, the forms separated by thermal lines. The form in the southern part of the country differs in the presence of perithecia, a slightly different acervulus, and entirely different cultural characters. Schneider-Orelli ('12) compared the American form of apple *Gloeosporium* with the European form and found that the former was distinguished from the latter by having the higher optimum and maximum temperatures and also a tendency to grow faster at the higher temperatures. Furthermore Edgerton ('15) noticed that Schneider-Orelli's American form might agree very closely with those of his so-called southern form, and also in all probabilities the European form might be identical with his northern form. It might not be unnecessary to state briefly the most important differentiating characters of these forms described by him. One form grows rapidly, has higher maximum and optimum temperatures, produces but scanty aerial mycelium in cultures, produces canker on apple limbs besides rotting the fruit, occurs principally in the southern warmer parts of the apple belt, and very commonly produces perithecia on the host as well as in cultures. According to him it seems probable that this fungus is identical with forms found on a number of hosts. If it was identical with the one found on *Ligustrum,* as seemed probable, it should be called *Glomerella cingulata.* The other

form grows slowly, has lower optimum and maximum temperatures, usually a greater abundance of white aerial mycelium on most culture media, apparently does not produce cankers on apple limbs though rots the fruit, occurs principally in the northern cooler sections, and apparently does not produce perithecia. It was also suggested that the name of *Gloeosporium fructigenum* seemed well to be used for this form. More recently Hemmi ('20) in his extensive studies on *Gloeosporium* noticed two distinct forms of apple anthracnose. The most important points of difference chiefly lie in temperature requirement, cultural characters and symptom of disease produced by those forms. Concerning cherry anthracnose Osterwalder ('04) remarked the similarity of the cherry anthracnose organism to the form of apple anthracnose, and suggested that it seemed well to use the name *Gloeosporium fructigenum* and also came in agreement with Southworth ('91) by believing that *Gl. fructigenum, Gl. laeticolor* and *Gl. versicolor* were not distinct species, but the names given to the same pathogen. According to Hemmi ('20) the form of cherry anthracnose seemed to be rather identical with that of tomato anthracnose. Suematsu and Kuwatsuka ('20), in comparing a peach anthracnose pathogen with other related species by extensive inoculation experiments, observed some distinctions between two forms from apple and cherry.

As briefly stated above, it may be rather difficult to determine whether forms of *Gloeosporium* found on those fruits represent a single species or more than one. A chief difficulty rests upon the fact that a culturable fungs exhibits considerable differences in morphological characters and physiological behaviors under the influence of changes in culture media or other environmental conditions. Especially this change, according to my studies on *Rhizoctonia* ('21 and '23) seems to be more striking when the fungus is grown for a time in different hosts. For the determination of the species, forms, or races, therefore, it is necessary to take into consideration all of the factors such as affect the growth and

the structure of the fungi, and a comparison should be attempted only after careful observation of physiological and morphological characters, accompanied by extensive inoculation experiments.

The chief object of the present paper is to make comparative studies with special referenc to physiological behaviors between the two original strains from apple and cherry, and also between those reisolated strains, the latters being obtained from the plants used in the inoculation experiments as will be described later.

Source of Materials

G.A. The material was obtained from a bitter rot of apple, var. Rawle's Janet, growing in our college orchard. Isolation was made from a dark brown sunken patch with numerous minute black points. Pulp of the fruit was also affected to a certain extent.

G.C. The material was obtained from a cherry, var. Napoleon Bigarreau, growing in our college orchard. Affected berries were perfectly dried and shrivelled, with pinkish mass of conidia covering the whole mummified surface. Isolation was made by transferring an affected tissue which was previously sterilized with corrosive sublimate and subsequently rinsed with sterilized water.

G.A. cherry I.* Isolated from a cherry inoculated with G.A.
G.A. cherry II.* do.
G.C. cherry I.* Isolated from a cherry inoculated with G.C.
G.C. cherry II* do.
G.A. apple.** Isolated from an apple inoculated with G.A.
G.C. apple.** Isolated from an apple inoculated with G.C.
G.A. pear.*** Isolated from a pear inoculated with G.A.
G.C. pear.*** Isolated from a pear inoculated with G.C.

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* See P. 41; ** see P. 43; *** see P. 45–46.

Cultural Characteristics

1. Synthetic culture media. It is generally recognized that the presence or absence of a certain element or compound and its concentration may closely be related to the growth, form and reproduction of parasitisms. Concerning the subject closely connected with my present investigation the work of Molisch ('94), Currie ('17) and Steinberg ('19) may be referred, but more recently Young and Bennet ('22) working with 21 species of parasitic fungi has shown that zinc might have a stimulating effect in the case of a limited number of the organisms, while in the case of some 15 species of organisms zinc has not accelerated the growth. By the same authors it has also been stated that in the majority of instances calcium proved to be rather beneficial. Concerning the subject, however, it might be rather difficult to give any conclusive data, because it is generally accepted that some of the elements are dissolved from the glassware or introduced from many of the purest salts to supply the need of the parasitic organisms. Therefore in the following experiments an emphasis is only placed on the study with special reference to the effect of some inorganic compounds under the conditions available at an ordinary laboratory equipment. The chemicals are obtained from the Merck Co., and they are placed in Erlenmeyer flasks of the highest grade. Duplicates or triplicates of all solutions are inoculated with each strain. Each dry weight of mycelium is read twice at different times, and an average number is taken for the result.

Experiment I. For this purpose the following standard solution is prepared; NH_4NO_3, 1 gm., KH_2PO_4, 0.25 gm., $MgSO_4$, 0.25 gm., peptone, 10 gms. and glucose, 20 gms. in 1000 cc. doubly distilled water. The arrangement of the experiment and the result after the incubation period of 3 weeks at 28°C. will be shown in table I. Fifty cc. of each of the solutions are placed in each flask, and each solution is set up in a triplicate.

TABLE I.

GROWTH OF THE STRAINS ON SYNTHETIC CULTURE MEDIA.

Culture				G.A.			G.C.		
No.	Standard sol'n, containing	pH	Original color of media	Dry weight of mycelium in gm.	pH	Remark	Dry weight of mycelium in gm.	pH	Remark
1	0.2% $Ca(NO_3)_2$	5.8	Pale orange yellow	0.366	8.6	Mycelial felt greenish white; solution capucine yellow; no sugar remained.	0.427	8.4	Mycelial felt pinkish in color; solution light orange yellow; no sugar remained.
2	0.2% KNO_3	5.6	,,	0.328	8.8	Mycelial felt greenish white; solution orange rufous; no sugar remained.	0.278	8.6	Mycelial felt pinkish in color; solution salmon orange; no sugar remained.
3	0.2% $NaNO_3$	5.5	,,	0.413	8.7	Mycelial felt greenish white; solution salmon orange; no sugar remained.	0.324	8.6	Mycelial felt pinkish in color; solution bitter-sweet orange; no sugar remained.

Experiment II. Standard solution is prepared by dissolving NH_4NO_3, 1 gm., KH_2PO_4, 0.25 gm., $MgSO_4$, 0.25 gm., peptone, 20 gms. and glucose, 20 gms. in 1000 cc. doubly distilled water. The arrangement of the experiment and the result after the incubation period of 3 weeks at 28°C. will be shown in table 2. Fifty cc. of each of the solutions are placed in each flask, and each solution is set up in a duplicate.

TABLE II.

SHOWING THE GROWTH OF THE STRAINS ON SYNTHETIC CULTURE MEDIA.

Culture			G.A.		G.C.	
No.	Standard sol'n, containing	pH	Dry weight of mycelium in gm.	pH	Dry weight of mycelium in gm.	pH
1	0.05% KNO_3	5.7	0.295	8.8	0.315	8.4
2	0.05% $NaNO_3$	5.7	0.320	8.8	0.335	8.6
3	0.05% KNO_3, eliminating peptone.	4.3	0.165	8.0	0.220	5.0
4	0.05% $NaNO_3$, eliminating peptone.	4.3	0.168	7.8	0.225	5.0

As shown by these experiments the presence of calcium nitrate has proved to be more beneficial for the growth of G.C. than any of the remaining compounds. Natrium nitrate has also proved to be a desirable food material, especially a considerable acceleration of growth is noticed in the cultures of G.A. Presence of KNO_3, however, has given the poorest result in both of the strains. Effect of natrium, however, is more sparingly noticed when peptone is eliminated from the standard solution. Change of pH value due to the growth of the mycelium is shifted toward alkaline side, and this shifting is more striking in the case of G.A.

Experiment III. The following standard solution is prepared; $NaNO_3$, 10 gms., KH_2PO_4 5 gms., $MgSO_4$, 2.5 gms. and glucose, 20 gms. in 1000 cc. of doubly distilled water. The result after the inculation period of 3 weeks at 28°C. will be shown in the following table. Forty five cc. of each of the solutions are distributed into each fiask. Each solution is set up in a duplicate. The solutions containing $FeCl_3$ present some precipitates when the salt is added to the standard solutions.

TABLE III.

GROWTH OF THE STRAINS, G.A. AND G.C. ON SYNTHETIC CULTURE MEDIA.

Cultrue			G.A.			G.C.		
No	Standard solution containing	pH	mycelial growth in gm.	pH	Remark	Mycelial growth in gm.	pH	Remark
1	0.05% KCl	4.3	0.160	7.4	Aerial hyphae abundant, mouse gray color; spore scarce; solution, cream buff.	0.110	7.4	Aerial hyphae scanty, pale pinkish; spore formed; solution light vinacious cinnamon.
2	0.05% $ZnCl_2$	4.3	0.080	4.5	Aerial hyphae very poor; solution hyaline.	0.235	7.6	Aerial hyphae medium in growth, pale pinkish; solution hyaline.
3	0.05% $FeCl_3$	3.4	0.085	5.2	Mycelium as before; solution cream buff.	0.200	7.8	Aerial hyphae medium in growth, pale pinkish; solution cream buff.
4	9.05% $CaCl_2$	4.4	0.190	8.2	Aerial hyphae abundant, white, but slightly grayish at the center of the mycelial felt; solution colonial buff.	0.195	8.0	Aerial hyphae medium in growth; solution pale pinkish buff.
5	Standard solution only	4.3	0.140	7.8	Mycelium mouse gray; solution ivory yellow.	0.185	8.0	Mycelium pinkish; solution light pinkish cinnamon.

Summarizing the result of table 3, calcium chloride is found to be beneficial for the growth of these 2 strains. Zinc chloride is desirable for G.C., but in the case of G.A. it strikingly retards growth. About the same tendency is noticed in the cultures containing ferric chloride. An addition of potassium chloride results in appreciable decrease in the growth of G.C., as compared with the cultures without it.

TABLE IV.

SHOWING THE GROWTH OF THE STRAINS ON SYNTHETIC CULTURE MEDIA.

Culture			G.A.			G.C.		
No.	Composition	pH	Growth in gm.	pH	Remark	Growth in gm.	pH	Remark
1	Standard sol'n	4.3	0.150	8.0	Mycelial growth rather fair; slightly grayish, solution pale yellowish.	0.095	2.8	Mycelial growth rather poor, slightly grayish; solution pale yellowish.
2	1/2 strength of the standard solution	4.5	0.068	8.0	Mycelial felt rather white, but darker in the mycelium contacting with the wall of the flasks; solution not discolored.	0.047	7.6	Mycelial felt slightly grayish; solution slightly yellowish.
3	1/4 strength of the standard solution	5.4	0.032	7.8	Mycelial felt mouse gray; solution not discolored.	0.035	7.4	About the same with No. 2, but no discoloration in solution.
4	The standard solution, plus 1% NH_4NO_3	4.2	0.138	7.4	About the same with No. 3, but solution slightly yellowish.	0.058	2.8	Growth very poor; solution slightly yellowish.
5	The standard solution, minus 0.5% NH_4NO_3	4.4	0.150	7.9	do.	0.128	6.6	About the same with No. 2.
6	The standard solution, minus 0.75% NH_4No_3	4.4	0.165	8.2	do.	0.167	7.0	Mycelial felt somewhat darker in color; spore produced.
7	The standard solution, plus 0.4% KNO_3	4.3	0.152	8.2	do.	0.112	2.8	About the same with No. 4.
8	The standard solution, plus 0.25% $MgSO_4$	4.3	0.152	8.2	do.	0.098	2.8	About the same with No. 7.

On the other hand, however, the same compound is found to be slightly beneficial for the growth of G.A. In all the caces

studied, the mycelial felt of G.A. has a tendency to become somewhat grayish, while the other pinkish in color. There are also some differences noticed in relation to the change of pH and color of the media at the end of the experiments.

Experiment IV. The following standard solution is prepared; KH_2PO_4, 0.5 gm., KNO_3, 4.0 gms., $MgSO_4$, 2.5 gms., NH_4NO_3, 10 gms., glucose 20 gms. and trace of ferrous sulphate in 1000 cc. doubly distilled water. The arrangement of the experiment and the result at the end of 3 weeks' incubation at 28°C. will be shown in table 4. Forty five cc. of these solutions are separately distributed into each flask. Each solution is set up in a duplicate.

As shown by the table the cultures of No. 1, 4, 7, and 8 inoculated with G.C. are shifted to acid side, while all the cultures inoculated with G.A. becoming alkaline at the end of the experiments. The diluted standard solutions are unsatisfactory for the growth of these two fungi. The standard solution, however, contains NH_4NO_3 too much to obtain the fair growth of our fungi, and a rather satisfactory result is obtained, when the cultures contain the salt at the concentration diluted to one fourth of the standard. An addition of KNO_3, or $MgSO_4$, to the standard solution gives no better result for the growth of the strains studied, with a single exception in the result of G.C. grown on the KNO_3-cultures.

Experiment V. The following standard solution is prepared; NH_4NO_3, 1 gm., KNO_3, 0.5 gm., KH_2PO_4, 0.25 gm., $MgSO_4$, 0.25 gm. and trace of $FeCl_3$ in 1000 cc. doubly distilled water. Fifty cc. of each of the following solutions are placed in each flask, and incubated at 27°C. for 18 days. The solution is set up in a duplicate.

Glycerin furnishes best to the growth of G.A., while the same compound seems to be less favorable for G.C., and the most satisfactory result of the latter is obtained on the media containing sucrose. The result on the glucose media is about the same, showing no apparent difference between two strains. Malic acid and peptone seem to be unsatisfac-

TABLE V.

SHOWING THE GROWTH OF THE STRAINS ON SYNTHETIC CULTURE MEDIA.

Culture media			G.A.			G.C.		
No.	Standard solu'n, containing	pH	Growth in gm.	pH	Remark	Growth in gm.	pH	Remark
1	2% sucrose	4.2	0.165	—	A greater part of the mycelium produced at the bottom of the flask ; solution hyaline, spore absent.	0.257	—	Aerial hyphae abundant, somewhat grayish ; spore scarce ; solution hyaline.
2	2% starch	4.4	0.190	6.4	Aerial hyphae greenish gray, abundant ; solution hyaline ; spore formed.	0.142	3.4	Aerial hyphae yellowish gray ; solution hyaline ; no spore formed.
3	2% glucose	4.1	0.215	—	Aerial hyphae greeunish gray, scarce, some produced at the bottom ; no spore formed.	0.210	—	Aerial hyphae grayish ; no spore formed.
4	2% malic acid*	4.2	0.072	9.0	Aerial hyphae white ; spore formed ; solution hyaline.	0.047	9.0	Solution pinkish ; spore formed ; aerial hyphae rather scarce.
5	2% glycerin .	4.0	0.352	8.0	Aerial hyphae grayish, solution slightly yellowish ; no spore formed.	0.165	3.0	Aerial hyphae scarce.
6	2% peptone	5.2	0.075	8.4	Mycelium abundant at the bottom.	0.115	8.5	Mycelium abundant at the bottom.

tory as a source of carbon for these strains. With respect to the change of pH value due to the growth of the strains some marked differences are noticed between the two strains. There are also considerable differences exhibited on some cultural characteristics with special reference to color and appearance of mycelial felt, spore formation, and discoloration of the solutions.

* Solution containing malic acid is adjusted with NaOH.

2. Plant Decoction. A. Fluid media. It is well known that in germination of seed we can distinguish some chemical changes taken place in food substances. For instance insoluble starches, oils, and proteins are converted by enzymes into soluble sugars, fatty acids, and peptones etc., then those digested food is ready for translocation, and use in the growing parts of the embryo. Therefore, it is logically to be expected that there might be some differences in nutritive value between the decoctions of seeds in resting stage and in germination.

For the experiments 100 gms. of the following materials are added to 1000 cc. water. In the case of germinated seeds the full weight of materials required is soaked with diluted formalin and immediately washed in sterilized water. The materials are then removed to a germinator and placed in 28°C. thermostat. After germinated they are added with a required amount of water and incubated at 50°C. for 2 hours. In the other case the procedure is only differed by using the materials not germinated. In each flask is placed 50 cc. of the solutions to be studied, and each solution is set up in a duplicate. All the cultures are incubated at 28°C.

TABLE VI.

GROWTH OF THE STRAINS ON PLANT DECOCTION FLUIDS.

Culture media				Inoculated material	Incubation period	Growth of mycel. in gm.	pH	Sugar, (Cc. 0.5% $KMnO_4$ in 2 cc. sol'n)	Remark
Material	pH	Color	Sugar, (Cc. 0.5% $KMnO_4$ in 2 cc. sol'n)						
Germinated soybean seeds	5.6	Pale orange yellow	1.20	G.A.	3 weeks	0.252	8.6	0.00	Solution Saccardo's umber
,,	,,	,,	,,	G.C.	,,	0.188	8.6	0.15	do.
Soybean seeds	6.4	,,	2.00	G.A.	,,	0.205	8.8	0.00	Solution ferruginous.
,,	,,	,,	,,	G.C.	,,	0.158	8.8	0 25	do.
Germinated barley (grains)	5.4	Clay color	3.00	G.A.	16 days	0.225	5.8	0.40	Mycelial felt wet, shell pink, mycelium at glass wall white, mingled with black mass of sclerotia-like hyphae ; solution cinnamon ; starch trace.
,,	,,	,,	,,	G.C.	,,	0.310	6.8	0.00	Aerial hyphae pale rhodonite pink to white ; mycelium at glass wall hellebore red, mingled with black mass of sclerotia-like hyphae ; solution orange rufous ; starch none.

Culture media				Inoculated material	Incubation period	Growth of mycel. in gm.	pH	Sugar. (Cc. 0.5% $KMnO_4$ in 2cc. sol'n)	Remark
Material	pH	Color	Sugar, (Cc. 0.5% $KMnO_4$ in 2 cc. sol'n)						
Barley (grains)	6 0	Pinkish cinnamon	0.25	G.A.	16 days	0.030	7.0	0.30	Aerial hyphae very scarce; solution orange cinnamon; starch trace.
,,	,,	,,	,,	G.C.	,,	0.070	7.6	0.00	Aerial hyphae scanty; solution vinaceous tawny; starch none.
Germinated oat (grains)	6.2	Wood brown		G.A.	3 weeks	0.110	7.6		Aerial hyphae white; solution apricot buff.
,,	,,	,,		G.C.	,,	0.095	7.6		Mycelial felt pinkish; solution cinnamon rufous.
Oat (grains)	6.6	Orange cinnamon		G.A.	,,	0.010	8.2		Aerial hyphae scanty; solution rufous.
,,	,,			G.C.	,,	0.015	8.2		do.
Germinated rice (grains)	6.2	Cream buff		G.A.	,,	0.010	6.0		Mycelial growth rather poor; solution pinkish buff.
,,	,,	,,		G.C.	,,	0.065	7.2		Mycelial hyphae pinkish; solution pinkish cinnamon.

Culture media									
Material	pH	Color	Sugar, (Cc. 0.5% $KMnO_4$ in 2 cc. sol'n)	Inoculated material	Incubation period	Growth of mycel. in gm.	pH	Sugar. (Cc. 0.5% $KMnO_4$ in 2 cc. sal'n)	Remark
Rice (grains)	6.6	Pinkish cinnamon		G.A.	3 weeks	0.010	7.8		Mycelial growth very poor.
,,	,,	,,		G.C.	,,	0.015	7.8		do.
Apple	4.0	Light buff		G A. apple	,,	0.098	4.1		Mycelium abundant at the bottom ; aerial hyphae light gelandine green ; solution warm buff.
,,	,,	,,		G.C. apple	,,	0.162	8.2		Aerial hyphae glaucous gray, solution apricot orange.
,,	,,	,,		G.A. cherry I.	,,	0.178	7.0		Aerial hyphae light gelandine green, some at the bottom ; solution antimony yellow.
,,	,,	,,		G.C. cherry I.	,,	0.188	8.1		Aerial hyphae glaucous gray ; spore abundant ; solution rufous
,,	,,	,,		G.A. cherry II.	,,	0.235	7.9		Aerial hyphae artemisia green ; solution warm buff.

Culture media				Inoculated material	Incubation period	Growth of mycel. in gm.	pH	Sugar, (Cc. 0.5% $KMnO_4$ in 2 cc. sol'n)	Remark
Material	pH	Color	Sugar, (Cc. 0.5% $KMnO_4$ in 2 cc. sol'n)						
Apple	4.0	Light buff		G.C. cherry II.	3 weeks	0.177	8.1		Aerial hyphae glaucous gray; solution apricot orange.
Corn grains				G.A.	"	poor			No discoloration in solution; starch abundantly remained; spore formed very scarcely.
"				G.C.	"	fair			Solution pinkish; starch almost exhausted; spore formed in abundance.

As shown by these experiments it is evidently confirmed that these two strains exhibit some distinctive and rather constant specializations, and could not be included under a single form of pathogen. In any cases the decoctions of the germinated seeds are more favorable for the growth of our fungi than those of the seeds at resting stage.

B. Solid media. Plant decoction is prepared in the manner described by Duggar, Severy and Schimitz ('17).

TABLE VII.

GROWTH OF THE STRAINS ON SOME PLANT DECOCTION AGAR.

Media	Strain	Remark
Carrot	G.C. cherry I.	Aerial hyphae medium well developed, white, but pinkish discoloration developed in the mycelium contacting with the wall of the tubes; numerous minute pinkish pustules of conidia on the surface.
,,	G.A. cherry I.	Aerial hyphae well developed, compact, grayish, deep grayish discoloration developed in the mycelium contacting with the glass wall and substratum; pinkish pustule of conidia very scarce.
,,	G.C. cherry II.	Similar to G.C. cherry I.
,,	G.A. cherry II.	Similar to G.A. cherry I.
Potato	G.C.	Aerial hyphpe poorly developed, pinkish; pinkish pustule of conidia rather abundant.
,,	G.A.	Aerial hyphae medium, grayish; scanty spore formation.
Germinated soybean	G.C.	Aerial hyphae having a tendency to grow erect, abundant, white, numerous minute black spots produced, accompanied by pinkish pustules of conidia; pinkish discoloration developed in the mycelium contacting with the glass wall and substratum.
,,	G.A.	Aerial hyphae very abundant, white, very compact, no black mass of mycelium developed; conidia very scarcely produced.
Soybean	G.C.	Similar to G.C. on germinated soybean.
,,	G.A.	Similar to G.A. on germ. soybean.
Apple	G.C.	Aerial hyphae well developed, erect, rather loose, shaded to pink in color, with pinkish pustules of conidia and blackish minute mass of sclerotia-like hyphae.
,,	G.A.	Aerial hyphae very well developed, compact, white, shaded to greenish gray in old cultures; no noticeable spore-oozes nor black spots developed; grayish black discoloration developed in the mycelium contacting with the substratum.
Malt	G.C.	Aerial hyphae grayish, erect, conidia ooze out in pinkish mass; pinkish discoloration developed in the mycelium contacting with the substratum.

Media	Strain	Remarks
Malt	G.A.	Aerial hyphae very compact, white or somewhat greenish gray; no spore ooze; no marked discoloration appeared in the mycelium contacting with the substratum.
Radish	G.C.	Aerial hyphae well developed, more or less errect, white, shaded to pinkish color; spore formation rather scanty.
,,	G.A.	Aerial hyphae medium well developed, shaded to greenish gray.
Potato	G.C. apple	Similar to G.C. on potato.
,,	G.A. apple	Similar to G.A. on potato.
Radish	G.C. apple	Similar to G.C. on radish.
,,	G.A. apple	Similar to G.A. on radish.
Potato	G.C. pear	Similar to G.C. on potato.
,,	G.A pear	Similar to G.A. on potato,
Radish	G.C. pear	Similar to G.C. on radish.
,,	G.A. pear	Similar ta G.A. on radish.

As shown by these experiments it is to be inferred that these two races show more or less distinctive characteristics under any particular set of conditions, and some of these characteristics seem to be rather constant under the influence of changes in the culture media or transfer to host.

Effect of H-ion Concentration on Mycelial Growth and Enzyme Activity.

Experiment 1. Paisin decoction is prepared by using 70 gms. of seedless raisin in 500 cc. doubly distilled water. A range of pH value and the result after the growth period of 3 weeks at 27°C. will be tabulated as follows. Twenty five cc. of each of the solutions are placed in 150 cc. Erlenmeyer flasks, and each solution is set up in a duplicate.

TABLE VIII.

SHOWING A RANGE OF pH VALUE AND THE EFFECT OF H-ION CONCENTRATION ON THE MYCELIAL GROWTH OF THE STRAINS.

Culture media						G.A.	G.C.
No.	N/5NaOH	N/5HCl	Solution	H_2O	pH	Dry weight of mycelium in gm.	Dry weight of mycelium in gm.
1	0	10.	50	40.0	2.0	0	0
2	0	7.0	,,	43.0	2.7	0	0
3	0	4.0	,,	46.0	3.3	0.159	0.186
4	0	1.0	,,	49.0	3.8	0.209	0.169
5	0	0	,,	50.0	4.2	0.209	0.189
6	2.5	0	,,	47.5	5.2	0.192	0.169
7	3.5	0	,,	46.5	6.3	0.189	0.179
8	4.2	0	,,	45.8	7.0	0.207	0.180
9	5.0	0	,,	45.0	8.2	0.201	0.199
10	7.5	0	,,	42.5	9.3	0.181	0.197

The above mentioned results show that the maximum growth for the strain G. A. is obtained on the media having pH 3.8-4.2, and the second maximum is also noticed in a growth curve at pH 7.0. This is similar to the results of Webb('19) and Hopkins('22) in their work concerning the effect of H-ion concentration on the growth of certain parasitic fungi. On the other hand, however, the growth of the strain G. C. is rather irregular, having two minimum points in a growth curve, pH 3.8 and pH 5.2 respectively, and so far as the present experiments are concerned, the maximum growth is obtained at an alkaline side.

Experiment II. A solution is prepared by dissolving KNO_3,2.00 gms, $MgSO_4$,0.5 gm., KH_2PO_4,0.1 gm., and glucose 20 gms. in 500 cc. doubly distilled water. A range of pH value and the result after the growth period of 2 weeks at 27°C. will be shown in the following table. Twenty

five cc. of each of the solutions are placed in Erlenmeyer flasks, and each solution is set up in a duplicate.

TABLE IX.

SHOWING A RANGE OF pH VALUE AND THE EFFECT OF H-ION CONCENTRATION ON THE MYCELIAL GROWTH OF THE STRAINS.

Culture						G.A.	G.C.
No.	N/5NaOH	N/5HCl	Solution	Water	pH	Dry weight of mycelium in gm.	Dry weight of mycelium in gm.
1	0	2	60	58.0	2 8	0.075	0.078
2	0	1	,,	59.0	3.2	0.075	0.071
3	0	0.5	,,	59.5	3.6	0.080	0.079
4	0	0.2	,,	59.8	4.1	0.085	0.083
5	0	0.1	,,	59.9	4.5	0.085	0.073
6	0	0	,,	60.0	5.0	0.080	0.075
7	0.1	0	,,	59.9	5.5	0.080	0.071
8	0.2	0	,,	59.8	6.2	0.082	0.072
9	0.5	0	,,	59.5	6.6	0.070	0.068
10	1.0	0	,,	59.0	8.1	0.070	0.070

A somewhat similar result in relation to H-ion concentration is noticed in this experiment. The maximum and the second maximum of the strain, G. A, is noticed at pH 4.1 and pH 6.2 respectively, while the growth of the strain G. C. is rather irregular, and so far as the present experiment is concerned no decisive data concerning the effect of H-ion concentration on the growth of G. C. can be obtained. In this experiment the maximum growth of the strain, G. C. is noticed at pH 4.1. As a whole, however, it is to be inferred that the growth of these two strains is not so strikingly effected by the change of H-ion concentration within the limits studied.

Experiment 3. The fungi have grown on the 50 cc. of the culture solutions containing NH_4NO_3,1 gm. ,KH_2PO_4,

0.25 gm., $MgSO_4$,0.25 gm., $NaNO_3$,1 gm., peptone 10 gms., and glucose 20 gms., in 1000 cc. doubly distilled water. After a growth period of 3 weeks at 25°C. the cultures are filtered through paper, and the filtrates are used for the study of diastase. Average dry weight of mycelium obtained from three flasks will be shown as follows: G.C.,0.315 gm., and G.A., 0.388 gm. A range of pH value is obtained according to the method described in the following table.

TABLE X.

AMOUNTS OF MATERIAL USED FOR PREPARING SOLUTIONS.

No.	Cc. of N/10 HCl	Cc. of N/10 NaOH	Cc. of Water	Cc. of 2% starch	Cc. of enzyme
1	1.8	0	12.2	10	6
2	1.2	0	12.8	,,	,,
3	0.6	0	13.4	,,	,,
4	0.2	0	13.8	,,	,,
5	0	0	14.0	,,	,,
6	0	0.2	13.8	,,	,,
7	0	0.6	13.4	,,	,,

To each of these solutions one per cent of toluol is added as an antiseptic. In the following table control A is set up by adding boild enzymes instead of natural enzymes, and control B is made by addition of doubly distilled water instead of starch solution. All the solutions are placed in a thermostat ranging from 29°to 30°C. At the end of 36 hours the hydrolysing power of the enzymes is determined by modified Bertrand method as described as follows. Two cc. of each of the solutions are accurately pipetted into each of two 15 cc. centrifuge tubes and made neutral to phenolphthalein by adding either NaOH or HCI. Five cc. of Fehling's solution are added to each of these tubes at a time, and the tubes are immersed immediately in an active boiling water

for 10 minutes. The amount of reduction is determined by using 0.5 % $KMnO_4$.

TABLE XI.

SHOWING THE ACTIVITY OF DIASTASE OF G.A. AND G.C.

No.	G.A.					G.C.				
	pH	Cc. of $KMnO_4$			Enzyme Activity	pH	Cc. of $KMnO_4$			Enzyme Activity
		Total	Control A.	Control B.			Total	Control A.	Control B.	
1	3.6	0 10	0	0	0.10	3.0	0.40	0	0	0.40
2	5.4	0.25	0	0	0.25	3.6	0.70	0	0	0.70
3	6.8	0.35	0	0	0.35	6.2	1.15	0	0	1.15
4	7.8	0.20	0	0	0.20	7.3	0.95	0	0	0.95
5	8.3	0.20	0	0	0.20	8.0	0.90	0	0	0.90
6	8.5	0.20	0	0	0.20	8.4	0.60	0	0	0.60
7	8.9	0.10	0	0	0.10	8.8	0.50	0	0	0.50

The experiment results in appreciable decrease in diastase activity of G. A. In this experiment a range of pH value is rather widely arranged, therefore no critical point for the maximum diastase activity of our fungi can be obtained, but so far as the present experiment is concerned the maximum activity for G. A. and G. C. is obtained at pH 6.8 and pH 6.2 respectively.

Experiment IV. Invertase activity of G.A. and G.C. is made according to the manner described in the preceding experiment. The result after the incubation period of 48 hours at 32°C. will be shown in the following table.

TABLE XII.

SHOWING THE ACTIVITY OF INVERTASE OF G.A. AND G.C.

No.	G.A.					G.C.				
	pH	Cc. of $KMnO_4$			Enzyme Activity	pH	Cc. of $KMnO_4$			Enzyme Activity
		Total	Control A.	Control B.			Total	Control A.	Control B.	
1	3.6	2.65	0.50	0	2.15	3.0	0.80	0.50	0	0.30
2	5.4	2.65	0.35	0	2.30	3.6	1.10	0.40	0	0.70
3	7.0	2.65	0.15	0	2.50	6.2	1.90	0.20	0	1.70
4	8.0	2.60	0.15	0	2.45	7.4	1.70	0.20	0	1.50
5	8 3	2.60	0.15	0	2.45	8.2	1.50	0.15	0	1.35
6	8.5	2.50	0.10	0	2.40	8.4	1.30	0.10	0	1.20
7	8.9	2.45	0.15	0	2.30	8.9	1.10	0.10	0	0.90

As shown by this experiment the invertase activity of G. A. is apparently much greater as compared with that of G. C., while in the diastase activity, as shown by the preceding experiment, an exactly contradictory relationship subsisting between these two strains. So far as the present experiment is concerned, the maximum invertase activity of G. A. and G. C. is noticed at pH 7.0 and pH 6.2 respectively.

Experiment V. Activity of maltase and emulsin is studied by using extracellular enzymes of the strains, G. A. and G. C., which have been grown on the solutions containing NH_4NO_3 1 gm., KH_2PO_4 0,25 gm., $MgSO_4$,0.25 gm., KNO_3 1 gm., peptone 10 gms., and glucose in 20 gms. in 1000 cc. water. The cultures after the growth period of 3 weeks at 25°C. are filtered through paper, and the filtrates are directly used for the study. Average dry weight of mycelium obtained from three flasks is read as follows: G. A 0.328 gm. and G. C. 0.278 gm. A rang of pH value is obtained in the manner described in the table 10, and the determination of the enzyme activity is made according to the method stated in experiment 3.

TABLE XIII.

SHOWING THE ACTIVITY OF EMULSIN OF G.A. AND G.C. AFTER THE INCUBATION PERIOD OF TWO DAYS AT 32°C.

No.	G.A.					G.C.				
	pH	Cc. of $KMnO_4$			Enzyme Activity	pH	Cc. of $KMnO_4$			Enzyme Activity
		Total	Control A.	Control B.			Total	Control A.	Control B.	
1	5.8	0.5	0	0	1.3	5.0	1.3	0	0	1.3
2	6.6	0.4	0	0	0.4	6.3	1.1	0	0	1.1
3	7.5	0.3	0	0	0.3	7.0	0.9	0	0	0.9
4	8.2	0.3	0	0	0.3	8.0	0.7	0	0	0.7
5	8.6	0.2	0	0	0.2	8.4	0.6	0	0	0.6
6	8.8	0 2	0	0	0.2	8.8	0.5	0	0	0.5
7	9.2	0.15	0	0	0.15	9.2	0.3	0	0	0.3

As shown by this table the emulsin activity is somewhat greater in the cultures grown by G. C. So far as the present experiment is concerned, the maximum activity of G. A. and G. C. is noticed at pH 5.8 and 5.0 respectively.

TABLE XIV.

ACTIVITY OF MALTASE OF G.A. AND G.C. AFTER THE INCUBATION PERIOD OF A WEEK AT 32°C.

No.	G.A.					G.C.				
	pH	Cc. of $KMnO_4$			Enzyme Activity	pH	Cc. of $KMnO_4$			Enzyme Activity
		Total	Control A.	Control B.			Total	Control A.	Control B.	
1	5.8	1.40	1.25	0	0.15	5.0	1.9	1 20	0	0.60
2	6.6	1.35	1.30	0	0.05	6.2	1.6	1.25	0	0.35
3	7.6	1.30	1.20	0	0.10	7.0	1.4	1.20	0	0.20
4	8.2	1.30	1.20	0	0.10	8.0	1.3	1.20	0	0.10
5	8.6	1.30	1.20	0	0.10	8.4	1.2	1.20	0	0
6	8.8	1.25	1.20	0	0.05	8.8	1.1	1.20	0	−0.10
7	9.2	1.25	1.20	0	0.05	9.2	1.2	1.20	0	0

Maltase is very sparingly detected in the cultures grown by G. A. So far as the present experiment is concerned the maximum activity of G. A. and G. C. is noticed at pH 5.8 and 5.0 respectively.

Experiment VI. Diastase and invertase activity of the fungi in question is studied with the use of mycelial extraction as well as several culture solutions on which the fungi have grown for three weeks at 30°C. Determination is made after the incubation period of 24 hours at 33°C.

TABLE XV.

DIASTASE AND INVERTASE ACTIVITY OF G.A. AND G.C

Enzyme	Source of enzymes	G.A.				G.C.			
		Cc. of $KMnO_4$			Enzyme Activity	Cc. of $KMnO_4$			Enzyme Activity
		Total	Control A.	Control B.		Total	Control A.	Control B.	
Diastase	Solution No. 1.*	0.20	0	0	0.20	0.60	0	0	0.60
	Solution No. 2.*	Negligible	0	0	Negligible	0.30	0	0	0.30
	Solution No. 3.*	do.	0	0	do.	0.50	0	0	0.50
	1% mycel. extraction	0.15	0	0	0.15	0.70	0	0	0.70
Invertase	Solution No. 1.*	2.35	0	0	2.35	0.75	0	0	0.75
	Solution No. 2.*	2.40	0	0	2.40	2.20	0	0	2.20
	Solution No. 3.*	2.40	0	0	2.40	2.20	0	0	2.20
	1% mycel. extraction	2.65	0	0	2.65	1.25	0	0	1.25

The results are exactly concordant with those shown in table 11 and 12.

* See experiment 1, (P. 19.)

Experiment VII. Comparative studies on the activity of diastase and invertase of the strains reisolated from some inoculated plants are also performed by using intracellular enzymes. Two grams of mycelial powder of each strain prepared from mycelium grown on raisin decoction for two weeks at 25°C. is added to 100 cc. of doubly distilled water, and they are kept at 32°C. for 12 hours, after which the solutions are filtered through paper. The process of the experiment is exactly similar to the method employed in the preceding cases.

TABLE XVI.

ACTIVITY OF DIASTASE AND INVERTASE OF THE STRAINS REISOLATED FROM SOME INOCULATED PLANTS.

Strain	Diastase at the end of 26 hours				Invertase at the end of 2 days			
	Cc. of $KMnO_4$			Enzyme activity	Cc. of $KMnO_4$			Enzyme activity
	Total	Control A.	Control B.		Total	Control A.	Control B.	
G.A.	0.25	0.05	0	0.10	2.35	0.35	0	2.00
G.C.	0.95	0.25	0	0.70	1.50	0.30	0	1.20
G.A. cherry I.	0.35	0.10	0	0.25	1.85	0.10	0	1.75
G.C. cherry I.	0.85	0.20	0	0.65	1.35	0.20	0	1.15
G.A. apple	0.30	0.10	0	0.20	2.15	0.20	0	1.95
G.C. apple	0.95	0.15	0	0.80	1.15	0.10	0	1.05
G.A. pear	0.40	0.10	0	0.30	2.00	0.10	0	1.90
G.C. pear	0.90	0.10	0	0.80	1.75	0.10	0	1.65

Summarizing the results it is inferred that each of our strains shows more or less distinctive and constant physiological characteristics with special reference to enzyme activity under the conditions arranged in the present investigations.

Inoculation

Experiment 1. Inoculation of Cherry Fruits. Inoculation experiments are begun on fruits of cherry trees, var. Governor wood, growing at the college orchard as soon as the blossoms begin to fall. Inoculations are made, on the one hand, by first pricking the surface of the fruits with a sharp sterilized needle and then placing a small bit of mycelium and conidia obtained from a pure culture, and, on the other hand, by directly inoculating fruits without prick. The inoculated fruits are immediately covered with a paraffin paper bag until the experiment is discontinued. The results of the experiments are tabulated as follows.

TABLE XVII.

INOCULATION OF CHERRY FRUITS.

Fungus	Size of fruits to be inoculated.	Method of inoculation.	Date of inoculation.	No. of fruits used.	No. of fruits infected.	Remark
G.A.	5 mm.	pricked	May 19	10	0	Observation was made in June 21. The inoculated fruits shrivelled, but presented no sign of disease.
G.A.	,,	not pricked	,,	,,	0	Observation was made in June 21. Some of them attained the full growth, but majority of them also shrivelled.
G.C.	,,	pricked	,,	,,	0	Observation was made in June 21. The inoculated fruits shrivelled, but presented no marked sign of disease.
G.C.	,,	not pricked	,,	,,	0	Observation was made in June 21. Some of them attained the full growth, but majority of them also shrivelled.
Control	,,	pricked	,,	,,	0	Observation was made in June 21. Majority of them shrivelled.

Fungus	Size of fruits to be inoculated.	Method of inoculation.	Date of inoculation.	No. of fruits used.	No. of fruits infected.	Remark.
G.A.*	8 mm.	pricked	May 25	10	4	Observation was make in June 12. Some of them shrivelled, but four of them produced a distinct lesion.
G.A.	,,	not pricked	,,	,,	0	Observation was made in June 12. A few of them shrivelled, but presented no sign of disease.
G.C.*	,,	pricked	,,	,,	5	Observation was made in June 12. A half number of them shrivelled, but the others produced a distinct lesion.
G.C.	,,	not pricked	,,	,,	0	Observation was made in June 12. A few of them shrivelled, but presented no sign of disease.
Control	,,	pricked	,,	,,	0	Observation was made in June 12. Majority of them attained the full growth, the others shrivelled.
G.A.*	attaining the full size, but still green in color.	pricked	June 6	,,	8	Observation was made in June 15. Pinkish pustules of conidia noticed. Observation in June 20: The lesion extended to half the surface of the fruits.
G.A.	,,	not pricked	,,	,,	2	Observation was made in June 20.
G.C.*	,,	pricked	,,	,,	9	Observation was made in June 15. Abundant pinkish pustules of conidia noticed. There is no marked difference in symptom between the infected fruits inoculated with G.A. and G.C.

* These reisolated strains are designated as G. A. cherry I, G. C. cherry I, G. A. cherry II, and G. C. cherry II respectively.

Fungus	Size of fruits to be inoculated.	Method of inoculation.	Date of inoculation.	No. of fruits used.	No. of fruits infected.	Remark.
						The lesion of the latter, however, has a tendency to sink and dry up somewhat more rapidly than the other.
G.C.	,,	not pricked	,,	,,	3	Observation was made in June 20.
Control	,,	pricked	,,	,,	1	It mignt be due to a natural infection.
G.A.	majority of them yellowish green but a few almost ripened.	pricked	June 12	,,	9	June 15: Slightly discolored, ca. 5 mm. in diameter. June 20: Spores not yet formed. June 21: Spore noticed.
G.A.	,,	not pricked	,,	,,	3	
G.C.	,,	pricked	,,	,,	10	June 21: Spores abundantly formed.
G.C.	,,	not pricked	,,	,,	2	
Control	,,	pricked	,,	,,	1	
G.A.	,,	pricked	June 14	20	18	June 21: Spores formed.
G.A.	,,	not pricked	,,	,,	4	
G.C.	,,	pricked	,,	,,	20	June 21: Spores formed.
G.C.	,,	not pricked	,,	,,	5	
Control	,,	pricked	,,	,,	3	

Summarizing the results it seems probable that the fungi can hardly attack the fruits when the latters are inoculated in very early growing season. So far as the present experiments are concerned the fungi seem to penetrate the fruits through uninjured surface and produce disease to a certain extent, but it is still left for the further investigation whether these lesions are actually caused by the artificial inoculations or due to natural infection through injured surface. As briefly stated in the table there is no marked specialization noticed in pathogenicity as well as in symptom produced by these two strains.

Experiment II. Inoculation of Apple Fruits. Inoculations are made on the fruits of apple trees, var. Rawle's Janet, growing at the college orchard. Inoculations are made by first pricking the surface of the fruits with a sharp sterilized needle and then placing a small bit of mycelium and conidia obtained from a pure culture of G.A. and G.C. The inoculated fruits are immediately covered with paraffin paper bag until the experiments are discontinued. Inoculation experiments are also conducted in the laboratory at irregular intervals through the season. In the latter case fruits to be inoculated are first sterilized with diluted formalin and then rinsed with sterilized water. Prior to inoculation each of them are placed in each sterilized tubulated glass-jar provided with a cotton-plugged hole at the top. Inoculation is made in the same manner as described in the preceding experiments. All the jars are placed in thermostat regulated at 25°C.

TABLE XVIII.

INOCULATION OF APPLE FRUITS.

Inoculated material	Size of fruit to be inoculated.	Place of investigation.	Date of inoculation.	No. of fruits used.	No. of fruits infected.	Remark.
G.A.	6–7 c.m	Orchard	Oct. 1	10	4	Lesion brown, spore very few.
G.A.*	,,	Laboratory	Oct. 1	10	10	Oct. 10: lesion 3 mm. Spores few. Development of disease is very slow.
G.C.	,,	Orchard	Oct. 1	10	2	Spores fairly produced.
G.C.*	,,	Laboratory	,,	10	10	Lesion strikingly sunken, 10 mm. Pinkish pustules of conidia very abundant. Development of disease is very rapid.

* These reisolated strains are designated as G.A apple and G.C. apple respectively.

Inoculated material	Size of fruit to be inoculated.	Place of investigation.	Date of inoculation.	No. of fruits used.	No. of fruits infected.	Remark.
Control	6–7 c.m	Orchard	Oct 1	10	0	
,,	,,	Laboratory	Oct. 1	10	0	
G.A.	,,	Orchard	Oct. 15	10	4	
G.A.	,,	Laboratory	,,	10	10	
G.C.	,,	Orchard	,,	10	3	
G.C.	,,	Laboratory	,,	10	10	
Control	,,	Orchard	,,	10	0	
,,	,,	Laboratory	,,	10	0	

Fruits inoculated with the strains present brownish discoloration at the infected point after the incubation period of 3 days, nevertheless the development of the disease infected by G. C. is strikingly faster than the other, and furthermore there is a marked difference in symptom developed from these two types of disease. The lesions produced by G. C. have a tendency to become shrunken rather extensively as compared with that produced by the other strain, and subsequently bear numerous pinkish pustules of conidia which are frequently oozed out, thus staining the affected surface with pinkish conidial mass. In the case of the other, however, the attacked surface does not sink so deeply and bear conidia rather scarcely as compared with the former.

Experiment III. Inoculation of Grape. Inoculations are conducted at the college orchard in the same manner as described in the preceding experiments. The result of the experiments will be tabulated as follows.

TABLE XIX.

INOCULATION OF GRAPE, VAR. CATAWBA.

Inoculated material.	Date of inoculation.	Method of inoculation.	No. of plant used.	No. of plant infected.	Remark.
G.A.	Sept. 21	pricked	20	4	Oct. 3: Symptom of the infected fruits is not a typical anthracnose. The remaining fruits almost ripened.
G.A.	,,	not pricked	20	0	Oct. 3: All the inoculated fruits attained the full vigorous growth and almost ripened.
G.C.	,,	pricked	20	5	Oct. 3: Symptom of the infected fruits is not a typical anthracnose.
G.C.	,,	not pricked	20	0	
Control	,,	pricked	20	0	

The fungi in question do not seem to cause a typical anthracnose on grapes, and no marked specialization in relation to grapes has been noticed in these two strains.

Experiment IV. Inoculation of Japanese Pear. Inoculations are conducted in the labaratory with the use of pears, var. Chojuro, which are treated in the same manner as described in experiment 2.

TABLE XX.

INOCULATION OF JAPANESE PEAR, VAR. CHOJURO.

Inoculated material.	Method of inoculation.	No. of plant used.	No. of plant infected.	Remark.
G.A.*	pricked	5	5	Observation was made after the incubation period of 2 weeks. The surface of the diseased spot slightly sinks, but spreads very slowly. Spores sparingly formed.

* These reisolated strains are designated as G.A. pear and G.C. pear respectively.

Inoculated material.	Method of inoculation.	No. of plant used.	No. of plant infected.	Remark.
G.A.	not pricked	5	0	
G.C.*	pricked	5	5	Observation was made after the incubation period of 2 weeks. Development of the disease is slightly faster than that of the former, and forming characteristic pinkish pustules of conidia on the brown patches.
G.C.	not pricked	5	0	
Control	pricked	5	0	

Discussion of data.

From all the evidences at hand it appears that our G.A. may be more or less closely related to Edgerton's slow-growing form as shown by contrast with his data.

Edgerton's slow growing strain.	G.A.
1. Usually produces a greater abundance of white aerial mycelium.	1. Aerial hyphae very compact, white, or in some instances slightly grayish.
2. Apparently does not produce cankers on apple limbs though it rots the fruit	2. So far as the present experiments are concerned, it does not produce cankers though rots the fruit.
3. Apparently does not produce perithecia.	3. No perithecia formation takes place.
4. Optimum temperature for growth, 25°C.	4. Optimum 23°–25°C.
5. Maximum temperature for growth, 35°, 5 C.	5. Maximum. Ca. 34°C.

Again, it may also be inferred that the strain seems to be identical with Hemmi's Apple, FG. II. in cultural characteristics as well as in temperature requirement, although no direct comparison could be made with his material. Considering the data noticed by Suematsu and Kuwatsuka in their extensive inoculation experiments, it seems probable that our G.C. may be identical with their cherry strain. No com-

parison could be made with Hemmi's Kirsche F. C. owing to insufficient data to be compared.

Concerning the relationship between our G.A. and G.C., there are marked differences in physiological characteristics, as shown by nutritive requirements, by color of mycelial felt, by enzyme activities and by pathogenicity. Furthermore these characteristics, so far as the present experiments are concerned, are rather constant, and are scarcely modified by the change of hosts or other environmental conditions. Nevertheless these differences alone may not perhaps be considered sufficient to distinguish these permanently as different species, because there are no apparent distinctions in morphological characteristics and also no marked specializations in pathogenicity to such an extent as to distinguish them as different species.

These evidences make it rather safe to assume that these two strains G. A. and G. C. may be regarded as two physiological forms of *Gloeosporium fructigenum.*

Summary

1. Acceleration of growth of our two strains is noticed when calcium nitrate, or calcium chloride is added to the standard nutrient solutions. The same is true with natrium nitrate.

2. Zinc chloride is desirable for G. C., but in the case of G. A. it strikingly retards growth. Almost the same tendency is observed in the cultures containing ferric chloride.

3. The solution having 1 % NH_4NO_3 0.05 % KH_2PO_4, 0.4 % KNO_3, 0.25 % $MgSO_4$ and 2 % glucose contains ammonium nitrate too much to obtain the fair growth of our fungi, and a rather satisfactory result is obtained, when the cultures contain the salt at the concentration diluted to one fourth of the standard. An addition of KNO_3, or $MgSO_4$ gives no better result for the growth of the strains studied,

with a single exception in the result of G. C. grown on the KNO_3-cultures.

4. Glycerin furnishes best as a source of carbon compound to the growth of G. A., while the same compound seems to be less favorable for G.C., and the most satisfactory result of the latter is obtained on the media having sucrose. The result on glucose media is about the same, showing no apparent difference between two strains. Malic acid and peptone seem to be unsatisfactory as a source of carbon for these strains.

5. The decoctions of the germinated seeds are more favorable for the growth of our fungi than those of the seeds at resting stage.

6. Concerning the effect of H-ion concentration on the growth of G. A. the minimum point in the curve is noticed. This is similar to the results obtained by Webb ('19) and Hopkins ('22).

7. Comparative studies on the activities of enzymes, i.e. diastase, invertase, maltase and emulsin, are made to a certain extent, and some marked specializations are noticed.

8. There are also considerable differences exhibited on pathogenicity as well as on some cultural characteristics with special reference to color and appearance of mycelial felt, spore formation, and discoloration and change of pH of the culture solution due to the growth of the fungi.

9. The specializations manifested by these two strains are rather constant, and seems to be least modified by the change of hosts or other environmental conditions.

10. Summarizing the results it is to be inferred that our two fungi can not be regarded as a single strain, but may properly be considered as two physiological forms of *Gloeosporium fructigenum*.

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Explanation of Plate.

Fig. 1. G.A, and G.C, grown on apple agar.
(1) From above: Left, G.A; right, G.C.
(2) From below: Left, G.A; right G.C.

Fig. 2. G.A, apple and G.C, apple grown on raisin agar.
(1) From above: Left, G.A, apple; right, G.C, apple.
(2) From below: Left, G.A, apple; right, G.C, apple.

Fig. 3. G.A, cherry 1 and G.C, cherry 1 grown on raisin agar.
(1) From above: Left, G.A, cherry 1; right, G.C, cherry 1.
(2) From below: Left, G.A, cherry 1; right, G.C, cherry 1.

Fig. 4. G.A, pear and G.C, pear grown on raisin agar.
(1) From above: Left, G.A, pear; right, G.C, pear.
(2) From below: Left, G.A, pear; right, G.C, pear.

Fig. 5. Inoculation of cherries, conducted at the laboratory.
(1) Result at the end of a week: Fruits above the paper, inoculated with G.A; fruits below the paper, inoculated with G.C.
(2) Result at the end of 10 days: Fruits above the paper, inoculated with G.A; fruits below the paper, inoculated with G.C.

Fig. 6. Inoculation of apples, conducted at the laboratory.
Left: Inoculated with G.A.
Right: Inoculated with G.C.

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